bjh research paper

Peripheral blood involvement in patients with follicular lymphoma: a rare disease manifestation associated with poor prognosis

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Received 3 August 2013; accepted for publication 18 September 2013

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Summary

Follicular Lymphoma (FL) is the second most common non-Hodgkin lymphoma (NHL) subtype and its course is heterogeneous. At diagnosis, some patients with FL manifest a detectable leukaemic phase (FL-LP), but this feature has been seldom described and is poorly characterized. Among 499 patients diagnosed with FL in Lyon-Sud hospital, 37 (7·4%) had characteristic FL-LP (by cytological blood smears and flow cytometric analysis). In addition, 91/1135 FL patients from the PRIMA study presented FL-LP at study entry. In order to evaluate the outcome of this Lyon-Sud cohort, FL-LP patients were matched with 111 newly diagnosed FL without LP according to the Follicular Lymphoma International Prognostic Index (FLIPI) score, age and treatment. Presence of FL-LP was associated with shorter progression-free survival (PFS) and overall survival (OS) ($P = 0{\cdot}004$ and $P = 0{\cdot}031$, respectively). Presence of FL-LP and high FLIPI score remained independent prognostic factors in a Cox model for time to progression (TTP). A number of circulating lymphoma cells (CLC) $>4 \times 10^9$/l was the most significant predictor for a shorter TTP in this Cox model. The prognostic impact of FL-LP on TTP was validated in the PRIMA cohort ($P = 0{\cdot}0004$). In conclusion, FL-LP is a rare event associated with shorter PFS and patients with CLC $>4 \times 10^9$/l have a poorer outcome. These patients should be monitored carefully to consider alternative therapeutic options.

Keywords: follicular lymphoma, peripheral blood involvement, prognostic factor, rituximab maintenance.

Follicular Lymphoma (FL) is the second most common non-Hodgkin lymphoma (NHL) subtype and the most frequent of the indolent NHL. It is an heterogeneous entity with some patients presenting an indolent clinical course for one or two decades, and others developing a much more aggressive disease and having a shorter survival (Campo *et al*, 2008). FL cells proliferate in the germinal centre and are characterized by the t(14;18) or one of its variants, present in almost 85% of cases, resulting in overexpression of the *BCL2* oncogene. The Follicular Lymphoma International Prognostic Index (FLIPI) and the FLIPI2 (Solal-Céligny *et al*, 2004; Federico *et al*, 2009) are used in clinical practice but fail to identify patients with a truly poor prognosis. While the FLIPI index is an accurate tool in the rituximab era (Relander *et al*, 2010), several prognostic factors are not considered, such as tumour bulk, serum level of β_2-microglobulin or blood involvement. Indeed FL often involves spleen and bone marrow (BM) but, in contrast to other indolent NHL at diagnosis, very few patients present with an overt detectable leukaemic phase (FL-LP). While a few reports described this finding (Melo *et al*, 1988; Chubachi *et al*, 1993; Hsieh *et al*, 2006; Rymkiewicz *et al*, 2006; Ganguly, 2009; Al-nawakil *et al*,

First published online 26 November 2013
doi: 10.1111/bjh.12675

2011), the prognostic impact of this phenomenon has not previously been evaluated. The aim of this study was to describe the clinical features of this population and its outcome. Analysis was performed in a retrospective patient cohort from the Lyon Sud Hematology Department (Hospices Civils de Lyon) and then validated in patients accrued in the PRIMA (Salles *et al*, 2011) study, a randomized open label study conducted by the Groupe d' Etude des Lymphomes de l' Adulte (GELA) between December 2004 and April 2007.

Patients and methods

Selection of patients

Among 499 patients diagnosed with FL according to the World Health Organization (WHO) criteria (Campo *et al*, 2008) (transformation to diffuse large B-cell lymphoma [DLBCL] and grade 3b FL excluded) in Lyon-Sud Hospital between January 1992 and January 2012, 37 cases (7·4%) had characteristic FL-LP detected by cytological blood smear analysis and confirmed by flow cytometry at time of diagnosis (identified on the basis of κ/λ monoclonality). All FL patients had an initial clinical examination and blood tests, including specific blood smears to look for circulating lymphoma cells (CLC), BM biopsy or aspirate, and computerized tomography (CT) body scan for staging. All data recorded in patients' files were collected retrospectively and anonymously coded. For all patients, complete response (CR) was assessed using standard response criteria (Cheson *et al*, 1999), including BM biopsy and CT scan. Immunostaining on blood smears or flow cytometry were also routinely performed on peripheral blood to verify the clearance of CLC. The study was conducted according to the Hospices Civils de Lyon ethic institutional guidelines and in accordance with the declaration of Helsinki.

Pathological and biological analyses

Blood smears were centrally reviewed for the study. For flow cytometric analysis, whole peripheral blood ($n = 37$) was stained using 2, 3 or 6 combinations of directly conjugated monoclonal antibodies (details are provided in Data S1). The samples were analysed on a Coulter EPICS XL flow cytometer. Additionally, in a subset of patients ($n = 15$), the expression of adhesion molecules (CD62L, CD11a, CD18, CD44 and CD54) was analysed on lymph node (LN) samples and compared to that observed in LN samples of FL without LP ($n = 24$). The immunophenotyping and flow cytometric methods have been previously reported (Traverse-Glehen *et al*, 2008). Histopathology samples were centrally reviewed for the study and classified according to the WHO 2008 classification (Campo *et al*, 2008). Immunohistochemical stains on whole tissue sections were performed to confirm the diagnosis of FL and to evaluate the microenvironment. CD68 staining was classified as high or low according to CD68 cell count (≥or <15/high-power field). In BM biopsy, the percentage of lymphoma cell infiltration was estimated by counting.

Cytogenetic analysis was performed in 23/37 cases and, in four patients, the *BCL2* rearrangement was studied by fluorescence *in situ* hybridization (FISH) using the appropriate probes (either *IGH*/*BCL2* or dual color break-apart *BCL2* probes – Vysis, Downer's Grove, IL, USA). Chromosome studies were performed as previously reported (Callet-Bauchu *et al*, 1999).

Identification of patients with FL-LP in the PRIMA study database

In order to expand and potentially validate findings in a prospective cohort of patients with FL, we used data collected in the PRIMA study (Salles *et al*, 2011). The presence of CLC was a criterion collected prospectively and monitored in the case report form, but the specific modalities used for the documentation of this manifestation at study sites were not available. Hence, it was not possible to provide the distribution of circulating lymphoma number or to validate the 4×10^9/l threshold in this cohort.

Statistical analysis

Statistical analyses were performed using STATISTICA software version 7.1 or SASv9.02 (for PRIMA patients). Time to progression (TTP) was calculated from the time of first treatment initiation (watchful waiting was considered as a first treatment if decided after initial consideration of management options) to time of disease progression. In the PRIMA study, progression-free survival (PFS) was calculated from time of registration (time of first therapy initiation) to time of disease progression. Overall survival (OS) was calculated from the time of first treatment initiation to time of death from any cause. Response and progression were defined using the international response criteria (Cheson *et al*, 1999, 2007). Survival functions were estimated by the Kaplan-Meier method and compared by log-rank test. Cox regression analysis was performed to adjust for the effect of prognostic factors relevant in univariate analysis. Patients' characteristics were compared using the Chi-square test. In the cohort of 37 patients with a FL-LP, univariate analysis for TTP including the different biological and clinical initial characteristics was performed. To further evaluate the impact of the number of CLC, a threshold of 4×10^9/l, corresponding to the upper limit of the normal lymphocyte count, was chosen. *P*-value <0·05 was used to define statistical significance.

Results

Clinical and biological characteristics of Lyon-Sud patients

Initial characteristics of the 37 patients with FL-LP at diagnosis are described in Table I A, B and Table SI. The median

Table I. Clinical (A) and biological characteristics (B) at diagnosis, of the 37 patients with FL-LP.

Clinical characteristic	*N* = 37
Age, years, median (range)	58·3 (28–80)
>60 years	13
≤60 years	24
Sex (Male/Female)	13/24
FLIPI score	
Low	4
Intermediate	16
High	17
Splenomegaly	
No	14
Yes	23
ECOG-PS	
0–1	34
2 or +	3
B symptoms	
No	29
Yes	8
High Tumour Burden	
No	11
Yes	26
Nodal Involvement	
No	0
Yes	37
>4 nodal localizations	
No	12
Yes	25

Biological characteristic	*N* = 37
Anaemia (Hb <120 g/l)	
No	28
Yes	9
Thrombocytopenia (platelets <100 × 10^9/l)	
No	32
Yes	5
Lymphocyte count	
Median (×10^9/l) (range)	4 (1–130)
% of clonal cells (median, range)	60% (6–99%)
β2-microglobulin >3 g/l	
No	15
Yes	22
Lactate dehydrogenase > normal	
No	26
Yes	11
CLC: (×10^9/l) median (range), mean	1·95 (0·60–128·70), 12·79
≤4 × 10^9/l	22
4–10 × 10^9/l	9
>10 × 10^9/l	6

FLIPI, follicular lymphoma international prognostic index; ECOG-PS, eastern cooperative oncology group performance score; CLC, circulating lymphoma cells.

age was 58 years. All the patients presented with nodal involvement. Most of the patients had an intermediate (n = 16) or high (n = 17) FLIPI score and the large majority of them (n = 26) had a high tumour burden, according to GELA criteria, at diagnosis (Solal-Celigny *et al*, 1993). Nine patients had anaemia and 5 had thrombocytopenia (platelet count <100 × 10^9/l).

All the patients had a biopsy specimen except one patient diagnosed on blood smears, BM aspirate and ascitic fluid samples, confirmed by flow cytometry and the presence of t (14;18) on karyotyping. Among the 36 histological samples available, 33 were LN biopsies and three extra-nodal biopsies (one of BM, one bone and one liver) with therefore limited immunohistochemical data. The characteristics are shown in Table II. All the tumours were CD20, CD10 and BCL2 positive except two cases that were BCL2 negative but demonstrated a positive *IGH-BCL2* translocation by FISH. All biopsy samples showed a pattern of inter-follicular dissemination with CD20, CD10 and BCL2 positive cells outside of the follicles. Most of them (84%) had a low- or intermediate-MIB1 score (<10% or 10–50% respectively). There were a majority (89%) of grade 1–2 FL with low fibrosis score (85%). Four patients presented a marginal zone differentiation pattern but with the t(14;18) as a hallmark of FL in 3 of them. Regarding the T-cell microenvironment, CD4+ T cells were always more abundant than CD8+ T cells. A marked peri-follicular localization of the CD3+ T cells was observed in eight cases. The presence of macrophages was assessed by CD68 expression and classified as high (six cases) or low (26 cases). The follicular dendritic cells meshwork was dense in 17 cases and weak in 16 cases. The median estimate of BM infiltration for patients with FL-LP was 51% (range 10–90%).

Flow cytometry identified a monotypic CD10 positive B cell population (n = 29), expressing surface immunoglobulin M (15/37), G (9/37), A (1/37), M+D (3/37) and M+G (3/37) (heavy chain was absent in four cases and not determined in two cases) with monotypic kappa (20 cases) or lambda (16 cases) light chain restriction (lack of light chain expression in one case). The median count of CLC was 1·95 × 10^9/l (range, 0·60–129·00 × 10^9/l). Fourteen and six patients had CLC counts higher than 4 × 10^9/l and 10 × 10^9/l, respectively.

Cytogenetic data at diagnosis were available for 23 patients (21 by conventional cytogenetics and four by FISH). All but one expressed the t(14;18), or its t(18;22) variant, and *IGH-BCL2* fusion was found in the four FISH analyses. Many patients had a complex karyotype (16/21), four presented with del(6q), two del(17p), three del(10q), three dup*BCL2*, one had a tetraploid karyotype, three patients had anomalies on 9p (p16 and p21 loci), and three on 17q.

Treatment and outcome

All but two patients, who were managed with watchful waiting, were treated at diagnosis. Thirty-five patients were treated with chemotherapy at diagnosis, which included rituximab for 27 patients (most patients received rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone

Table II. Prognostic analysis in the matched population.

Univariate Analysis $N = 148$	Median TTP (months)	Median OS (months)	Cox Model 1: *P*-value and HR for TTP ($N = 120$)	Cox Model 2: *P*-value and HR for TTP ($N = 120$)	Cox Model: *P*-value and HR for OS ($N = 120$)
CLC	$P = 0{\cdot}0035$	$P = 0{\cdot}031$	$P = 0{\cdot}0085$		
No *versus* ($N = 111$)	89	NR	HR = 2·2		
Yes ($N = 37$)	29	NR	95% CI (1·45–4·77)		
CLC	$P = 0{\cdot}00012$	$P = 0{\cdot}0019$		$P = 0{\cdot}0006$	$P = 0{\cdot}046$
$\leq 4 \times 10^9$/l ($N = 133$) *vs*	89	NR		HR = 3·57	HR = 3·7
$>4 \times 10^9$/l ($N = 15$)	15	NR		95% CI (1·74–7·24)	95% CI (1·02–12·9)
FLIPI	$P = 0{\cdot}0013$	$P = 0{\cdot}0014$	$P = 0{\cdot}0034$	$P = 0{\cdot}037$	$P = 0{\cdot}049$
Low and intermediate ($N = 81$)	NR	NR	HR = 2	HR = 1·37	HR = 1·95
High ($N = 67$)	43·2	133	95% CI (1·46–3·69)	95% CI (1·01–2·4)	95% CI (1–3·79)
B2-microglobulin	$P = 0{\cdot}017$	$P = 0{\cdot}085$	$P = 0{\cdot}15$	$P = 0{\cdot}066$	$P = 0{\cdot}29$
<3 mg/l ($N = 79$)	88	NR	HR = 1·52	HR = 1·92	HR = 1·88
>3 mg/l ($N = 41$)	45	NR	95% CI (0·85–2·71)	95% CI (0·92–2·83)	95% CI (0·57–6·1)

HR, Hazard Ratio.

Univariate prognostic analysis for time to progression (TTP) and overall survival (OS) in the matched population. $N = 148$ patients, 37 with circulating lymphoma cells (CLC) and 111 without. In this population the presence of CLC, a high Follicular Lymphoma International Prognostic Index (FLIPI) score and a high β2-microglobulin level were associated with a shorter TTP. Survival was significantly shorter for patients with high FLIPI score or CLC at diagnosis, but was not impacted by high β2-microglobulin level.

In a Cox regression model for TTP, high FLIPI score, and presence of follicular lymphoma with a detectable leukaemic phase (FL-LP) remained independently associated with a shorter TTP. In a Cox regression model for OS, FLIPI score was the only significant predictive factor (data not shown). In a second Cox regression model for TTP, including CLC count (higher than 4×10^9/l *versus* absent or lower than 4×10^9/l) instead of CLC presence or absence as variable, CLC count $>4 \times 10^9$/l and FLIPI were two independent prognostic factors associated with a shorter TTP and OS.

(CHOP or CHOP-like regimen). The eight patients treated before 2003 (when rituximab became routinely available as a first-line therapy) received CHOP or CHVP regimen (including cyclophosphamide, etoposide, adriblastine and prednisone). The overall response rate (ORR) was 83% (29/35 patients) and the CR rate was 66% (18 patients reaching CR and five unconfirmed CR [CRu]), five patients had stable disease and one progressed during treatment. The median TTP was 28·9 months. Actuarial TTP and OS rates were 37% and 86% at 5 years and 31% and 68% at 10 years, respectively (Fig 1). Among 23 patients achieving a CR or a CRu, 11 relapsed (between 9 and 74 months from attainment of remission, median 18 months). Five of the six patients with a partial response (PR) relapsed (at 3, 4, 12, 13 and 31 months from attainment of remission).

Of the 22 patients with refractory disease ($n = 6$) or further lymphoma progression ($n = 16$), 12 received salvage therapy followed by autologous stem cell transplantation (ASCT). Their median TTP from diagnosis was 8·3 months while median TTP after ASCT has not yet been reached, with a 10-year TTP estimate of 68%. Considering all patients, at a median follow-up of 72 months, eight patients had died, six from a lymphoma-related cause, including four after histological transformation.

Regarding the two patients managed with watchful waiting, one died after 24 months of follow-up due to development of DLBCL transformation. The other patient is alive without treatment initiation with a follow-up of 6·7 years.

Prognostic factors in the 37 patients with FL-LP

To evaluate the impact of CLC count, the threshold of 4×10^9CLC/l, corresponding to the upper limit of the normal lymphocyte count, was chosen. In univariate analysis (Table SII), several characteristics were associated with a shorter TTP in this cohort, namely splenomegaly ($P = 0{\cdot}035$), presence of high tumour burden ($P = 0{\cdot}017$), CLC count ($>4 \times 10^9$/l, $P = 0{\cdot}026$) and β_2-microglobulin level (>3 mg/l, $P = 0{\cdot}036$). Exploratory multivariate analysis including all these variables identified splenomegaly (Hazard ratio [HR] = 3·17; $P = 0{\cdot}029$) and a CLC count $>4 \times 10^9$/l (HR = 3·3; $P = 0{\cdot}009$) as independent prognostic factors for TTP.

Patients presenting a high CD68 expression tended to have a shorter median TTP (7 months) than the others (43 months); this difference was not statistically ($P = 0{\cdot}2$) significant due to the low number of patients analysed.

Matched pair analysis for clinical and prognostic factors

To further evaluate the impact of FL-LP on TTP and OS, we performed a 1:3 matched pair analysis without replacement according to FLIPI score, age, treatment type (observation *versus* chemotherapy, with or without rituximab) and treatment period (before or after 2000) (Bland & Altman, 1994; Sorensen & Gillman, 1995). All 37 patients were successfully matched with 111 newly diagnosed patients presenting with FL without LP (Table SIII). In the 111 matched patients with FL,

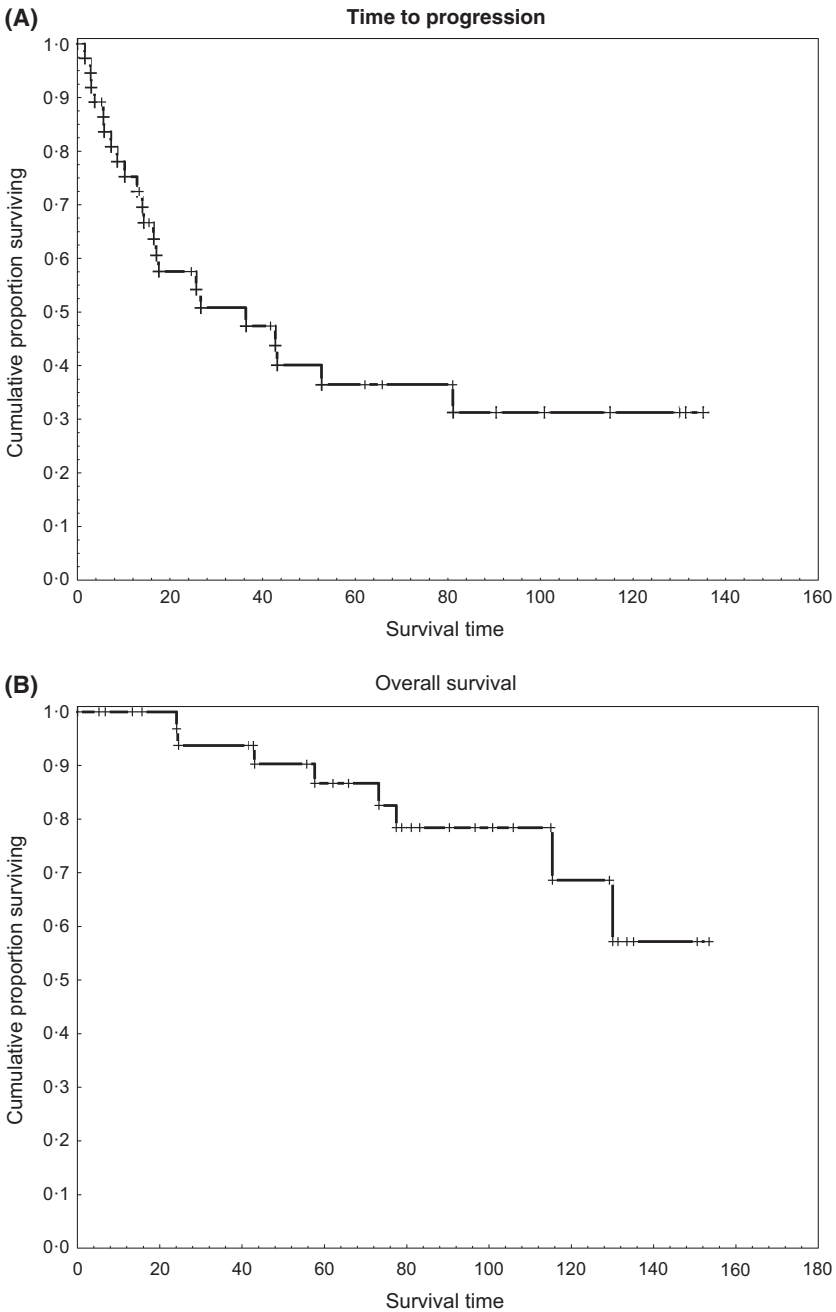

Fig 1. Time to progression and overall survival in the population of patients with FL-LP (A) Progression-free survival in the 37 patients presenting with follicular lymphoma with a detectable leukaemic phase (FL-LP) at diagnosis. Median time to progression (TTP) is 29 months. TTP estimates were 37% at 5 years and 31% at 10 years. (B) Overall survival in the 37 patients presenting with FL-LP at diagnosis. Overall survival estimates were 86% at 5 years and 68% at 10 years.

median TTP was 89 months with 5- and 10-year TTP estimates of 64% and 41%, respectively. Five- and 10-year survival estimates were 97% and 91%, respectively.

Prognostic analysis for TTP in the whole population of 37 patients with FL-LP and the matched 111 patients without FL-LP, including β_2-microglobulin level, FLIPI score and presence of FL-LP showed that high FLIPI score ($P = 0{\cdot}0013$), presence of FL-LP ($P = 0{\cdot}0035$) and high β_2-microglobulin level ($P = 0{\cdot}017$) were all significantly associated with an inferior outcome. In a Cox regression model for TTP including these three variables (performed in 120/148 patients with complete data), only high FLIPI score ($P = 0{\cdot}0034$; HR = 2) and the presence of FL-LP ($P = 0{\cdot}0085$; HR = 2·2) remained independently associated with shorter TTP (Table II). In a Cox regression model for OS, FLIPI score was the only significant predictive factor (data not shown). Similar results were found when patients under watchull waiting were excluded from the analysis (two in the FL-LP cohort and six in the matching cohort) (data not shown).

Comparison of pathological characteristics in the FL-LP and the matched population

Of the 111 patients without LP, 108 had a BM biopsy at diagnosis and 53 of those showed lymphomatous infiltration

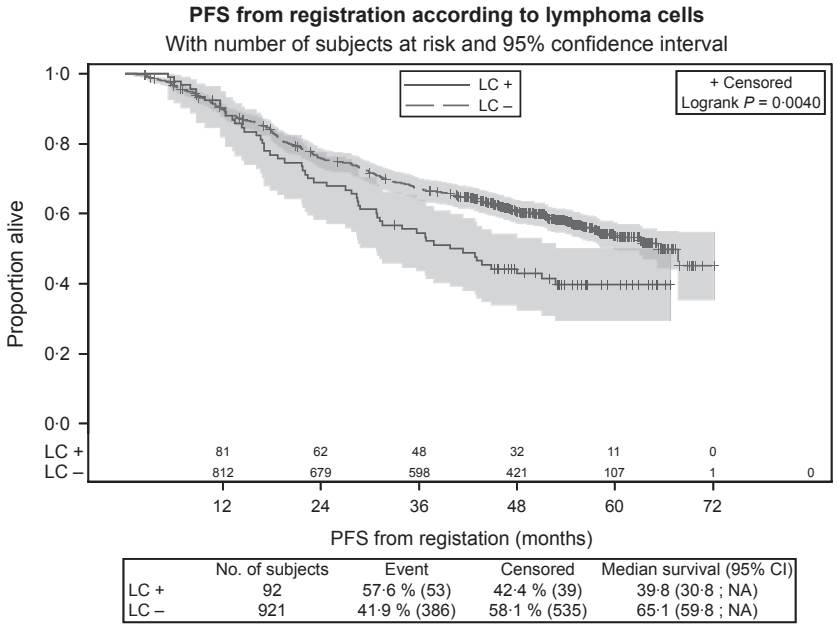

Fig 2. PRIMA study: Progression-free survival (PFS) from registration according to leukaemic phase At time of registration, 92 patients presented with follicular lymphoma with a detectable leukaemic phase (FL-LP) and 921 without. Patients with LP had a shorter PFS than the others. PRIMA: Primary RItuximab and Maintenance. ClinicalTrials.gov number NCT00140582 LC±: presence/absence of circulating lymphoma cells.

with a median infiltration rate of 15% (range from <5% to 70%). The median estimate of BM infiltration for patients with FL-LP was 51% (range 10–90%), which is significantly higher than the estimated infiltration of the 53 patients without LP ($P < 0{\cdot}0001$, Mann–Whitney *U*-test).

Additional flow cytometric analysis was performed on lymph nodes cells to evaluate the expression of adhesion molecules and results are detailed in Table SIV.

Validation on patients from the PRIMA study

To validate these findings in a prospective cohort of patients with FL, data collected from the PRIMA study were analysed (Salles *et al*, 2011). In the PRIMA trial, patients with previously untreated FL requiring systemic therapy were randomized after eight cycles of induction therapy (consisting of R-CHOP or R-CVP or R-FCM [rituximab, fludarabine, cyclophosphamide, and mitoxantrone] according to investigator preferences) between maintenance with rituximab for 2 years or observation. Among the population of 1135 patients with histologically-confirmed FL, 92 presented FL-LP (9%). After induction therapy they had the same ORR as patients without LP (ORR of 93·5% and 90·5% for patients with and without FL-LP, respectively, $P = 0{\cdot}455$). However, statistical analysis for PFS showed that FL-LP patients had a shorter PFS than those without LP when assessed from registration ($P = 0{\cdot}0004$, with a median PFS of 39·8 months *versus* 65·1 months) (Fig 2). No significant difference in terms of OS according to FL-LP was observed.

A heterogeneous population

To further analyse the impact of CLC on outcome, patients were categorized according to the number of CLC. The threshold of 4×10^9CLC/l, corresponding to the upper limit of the normal lymphocyte count, was chosen. Given the expected heterogeneity of CLC assessment in different PRIMA centres, this analysis was only performed on the Lyon-Sud retrospective cohort. As mentioned above, in the population of 37 patients with FL-LP, those with $>4 \times 10^9$/l CLC had a poorer outcome than those with $\leq 4 \times 10^9$/l. Interestingly, in the matched study, FL-LP patients with $\leq 4 \times 10^9$/l CLC had a similar TTP to those without (Figure S1) ($P = 0{\cdot}4$, median 66 and 89 months respectively). Furthermore, patients without FL-LP or with $\leq 4 \times 10^9$/l CLC had actuarial 5- and 10-year OS rates of 96% (standard error [SE] 1·7%) and 88% (SE 4·8%) vs. 73% (SE 13%) and 52% (SE 15·8%) for patients with $>4 \times 10^9$/l CLC, respectively ($P = 0{\cdot}0019$) (Fig 3). When CLC $>4 \times 10^9$/l (instead of CLC >0) was used as a cut-off point in the Cox regression model for TTP together with β_2-microglobulin and FLIPI score, the most significant predictor for a shorter TTP was CLC $>4 \times 10^9$/l ($P = 0{\cdot}006$; HR = 3·57) as compared to FLIPI score ($P = 0{\cdot}037$; HR = 1·37) and β2-microglobulin ($P = 0{\cdot}066$; HR = 1·92). In a Cox regression model for OS, CLC $>4 \times 10^9$/l ($P = 0{\cdot}046$; HR = 3·7) and FLIPI score ($P = 0{\cdot}049$; HR = 1·95) were also significant predictors (Table II).

Discussion

Although few reports include a substantial number of patients with FL-LP, this clinical characteristic was found in 7·4% and 9% of FL patients in a reference hospital department and in a prospective clinical trial recruiting only those patients with a high tumour burden. As described in the Lyon-Sud cohort, patients with LP presented an intermediate or high FLIPI score with a high tumour burden in

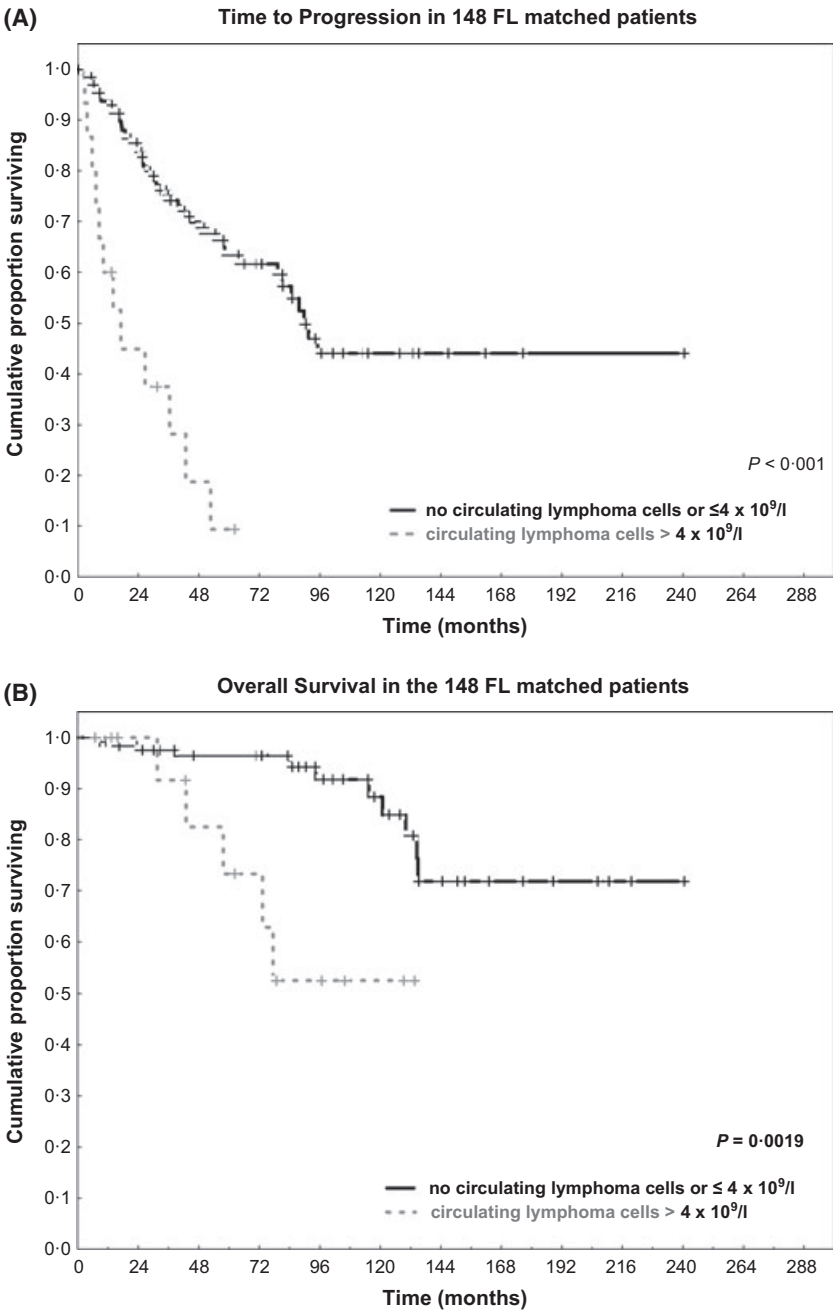

Fig 3. Time to progression (A) and overall survival (B) in the matched population of 148 follicular lymphoma (FL) patients according to circulating lymphoma cell (CLC) count (A) The 15 patients with>4 × 10^9/l of CLC at diagnosis have a worse median time to progression (15 and 89 respectively, $P = 0{\cdot}0001$) and overall survival ($P = 0{\cdot}008$) than the 133 patients with a CLC count ≤4 × 10^9/l. (B) Those with no detectable CLC or with a CLC count <4 × 10^9/l had a 5-year survival estimate of 96% (standard error [SE] 1·7%) and a 10-years estimate of 88% (SE 4·8%) vs. 73% (SE 13%) and 52% (SE 16%) for those with ≥4 × 10^9/l of CLC.

most cases. They also frequently had elevated β_2-microglobulin level. Multivarite analysis identified the presence of >4 × 10^9/l CLC as the most significant predictor independently associated with an inferior TTP in this population of patients with FL-LP.

Although retrospective, our series suggests that FL-LP is associated with a poor outcome. This observation was then confirmed in the PRIMA cohort. Interestingly, in this population with FL-LP, TTP after first relapse was longer than the first relapse-free interval (not shown), which might be explained by the frequent use of ASCT as salvage therapy. However, this population of FL-LP was not homogenous, and our data suggest that only patients presenting with >4 × 10^9/l CLC may have a significantly worst outcome. In our series, the prognostic value of a CLC threshold of 4 × 10^9/l on TTP and OS was stronger than the impact of established risk factors, such as the FLIPI score or β_2-microglobulin level, as shown by the Cox regression model performed in the matched population. To date in the literature, the largest study focusing on patients with FL-LP included 10 retrospective cases (Al-nawakil *et al*, 2011). Only six of these patients had concomitant LN involvement with a high tumour burden and four had pure FL-LP with a diagnosis relying on cytological examination and, for three of them,

cytogenetics or molecular biology. The authors suggested a more indolent clinical evolution for these patients without nodal involvement. The median follow up in the study reported by Al-nawakil *et al* (2011) was 26 months, one of the four patients was treated at diagnosis and one was lost to follow-up. Another study reported the useful prognostic value of high lymphocyte counts in FL but excluded patients with FL-LP (Siddiqui *et al*, 2006). Recently, many advances have been made in understanding the NHL microenvironment (Drillenburg & Pals, 2000; Gribben, 2010; Guilloton *et al*, 2012) and adhesion molecules have been shown to be highly implicated in lymphoma tissue-specific homing, dissemination and aggressiveness of the disease. Recent reports suggest that an increased serum level of cell adhesion molecules in soluble form, such as CD44, intercellular adhesion molecule 1 (ICAM1, CD54) or vascular cell adhesion molecule 1 (VCAM1, CD106), is associated with a worse outcome in aggressive lymphoma and in some indolent lymphomas (Niitsu & Iijima, 2002; Shah *et al*, 2012). The elevated serum level of CD54 is explained by proteolytic release of the molecule from the malignant cells' surface. Here the number of FL cases that expressed CD54 was lower in the FL-LP group. This suggests that, in FL-LP, cell surface CD54 could be cleaved and released as its soluble form, which would be consistent with the poorer prognosis of these patients. Recently, an enhanced proliferation rate and clonogenic capacity was found in cell lines with ectopic CD44 expression (Higashi *et al*, 2009; Hu *et al*, 2010). In our study, CD44 was also found to be more frequently expressed in patients with FL-LP.

Based on this data, patients with FL-LP at diagnosis should be considered as having a more aggressive disease course, relative to other indolent NHL. The same finding was also reported in DLBCL, where the prognostic value of LP has been recently associated with a worse outcome (Berger *et al*, 2000; Nodit *et al*, 2003; Rubio-Moscardo *et al*, 2005; Ondrejka *et al*, 2011; Muringampurath-John *et al*, 2012; Nygren *et al*, 2012). Although approximately one-third of the patients included in this series experienced long term TTP, all patients should be monitored carefully during and after first-line treatment to consider more intensive treatment, such as ASCT, when feasible as a therapeutic option to achieve a sustained response, especially if a complete response is not achieved or if their disease progresses shortly after their initial therapy.

Acknowledgements

We thank B. Bancel for expert technical assistance in immunohistochemistry and the LYSARC statistical department for the PRIMA-related analysis.

Conflict of interest

The authors report no potential conflict of interest.

Author contributions

GS, LB and CS designed the study. GS, CS, LK, LL, ASM, BC, OD, LB, CS, PF, AEG, FO, FS included patients in the study. GS and CS controlled the database. FB, LB, MF, PF, ATG, ECB reviewed biological data. GS, CS and JFS wrote the paper. All authors reviewed and approved the paper.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Detailed pathological and biological analyses.

Table SI. Histology and immunhistochemistry at diagnosis (36 patients with available biopsies).

Table SII. Univariate Prognostic analysis for TTP in the FL-LP population (Log Rank test).

Table SIII. Matched population.

Table SIV. complementary flow cytometry analysis on cell adhesion molecules.

Fig S1. TTP in in the matched population of 148 patients, according to CLC count.

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